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Regulation of Renal Sodium Calcium Exchange by PTH: Alteration with Age

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Parathyroid hormone, when incubated with renal cells acting *in vivo* and *in vitro*, increased Na^+/Ca^{2+} exchange activity. The effect of parathyroid hormone was specific for biologically active analogs and could be mimicked by cAMP and forskolin. Parathyroid hormone-sensitive Na^+/Ca^{2+} exchange activity was markedly blunted in cells from senescent rats. Parathyroid hormone-stimulated adenylate cyclase was also decreased in aging. In contrast, forskolin-stimulated Na^+-dependent Ca^{2+} efflux and adenylate cyclase did not change with senescence. Decrease of PTH binding sites was observed in cells from old rats. Further, cells from 24-month-old rats had decreased Gs and Gi proteins, as detected by ADP-ribosylation. Since serum iPTH level was elevated in the old rat and could contribute to the desensitization to PTH, we tested this hypothesis by comparing sham-operated and PTX animals. The decreases in PTH-sensitive Na^+/Ca^{2+} exchange activity and adenylate cyclase activity in cells from 24-month-old rats could be completely negated by parathyroidectomy. Decrease in PTH binding sites and contents of Gs and Gi in cells from aged-rats was partially negated by the surgery. In conclusion, our results suggested that the age related blunting in responses of renal cells to PTH was due, at least in part, to the elevated serum iPTH level in old rats.

Introduction

The Na^+/Ca^{2+} exchange system in the plasma membrane plays a key role in the extrusion of cellular Ca^{2+} and the control of the cytosolic Ca^{2+} concentration (*1*). In the kidney, the cytosolic free Ca^{2+} concentration is in the submicromolar range (*2*), whereas plasma and filtrate Ca^{2+} concentrations are about 2.5 mM. In addition, there is a negative membrane potential of 60 mv from exterior to interior of the cell. It is generally assumed that Ca^{2+} enters the tubular cell at the luminal membrane by a diffusional mechanism, but it has to be transported actively out of the cell at the basolateral membrane against both a chemical gradient and a membrane potential. Two distinct transport systems localized in the basolateral membrane have been proposed to engage in active exclusion of Ca^{2+}: one is a high-affinity Ca^{2+}-ATPase that serves as a pump (*3-5*); the other is the Na^+/Ca^{2+} exchanger that is driven by an inward Na^+ gradient.

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Effect of PTH *In Vivo* on Na^+/Ca^{2+} Exchange

Parathyroid hormone (PTH) has long been known to stimulate renal Ca^{2+} reabsorption (*6,7*). PTH receptors and adenylate cyclase have been shown to be localized in the basolateral membrane (*7-10*). Recently, we reported that Na^+/Ca^{2+} exchange activity is decreased by 40% in the basolateral membrane prepared from thyroparathyroidectomized rats and that the activity can be restored by infusion of PTH (*11*). In studies with dogs (*12*), it was found that the apparent V_{max} is altered with no change in the apparent K_m for Ca^{2+}. These findings clearly showed that PTH administered *in vivo* affects Na^+/Ca^{2+} exchange activity. However, these studies did not establish that the exchange activity in renal cells is directly affected by PTH. Therefore, we examined the effects of PTH when incubated with isolated rat cortical renal cells *in vitro*.

Effect of PTH *In Vitro* on Na^+-Dependent Calcium Efflux

Renal cells were preloaded with $^{45}CaCl_2$ for 30 min. To study the effect of PTH, PTH (10 U/mL) was added to the cell suspension 1.5 min before the end of

loading period. Efflux was initiated by dilution with a medium containing EGTA and with NaCl or choline chloride. The efflux time was 5 sec. As shown in Figure 1, the efflux of $^{45}Ca^{2+}$ was very rapid. In control cells (-PTH), the efflux with Na^+ in the extracellular medium was 17.6 ± 4.6%. This Na^+-dependent $^{45}Ca^{2+}$ efflux was stimulated 55% after exposure of the cells to PTH. However, the Na^+-independent $^{45}Ca^{2+}$ efflux was not affected by the hormone.

Other experiments indicated the specificity of the action of PTH. Equivalent units (10 U/mL) of the synthetic PTH (*1-34*) resulted in an increase of Na^+-dependent $^{45}Ca^{2+}$ efflux comparable to that found with PTH (*1-84*). In contrast, the inactive form of the hormone PTH (*3-34*) did not significantly affect Na^+-dependent $^{45}Ca^{2+}$ efflux. Cyclic AMP analogs, dibutyryl cAMP (1 mM), and 8-bromo cAMP (0.2 mM) also stimulated Na^+-dependent $^{4}Ca^{2+}$ efflux to the same extent. Forskolin, an activator of adenylate cyclase (*12*), also increased Na^+-dependent $^{45}Ca^{2+}$.

Effect of Age on the Sensitivity of Na^+-Dependent $^{45}Ca^{2+}$ Efflux to PTH

Calcium homeostasis is a critical problem in the aging animal. Increased level of immunoactive PTH was reported in the senescent rat (*13*). However, accumulation of cAMP in response to PTH was found to be decreased in renal slices from 12-month-old rats in comparison to 2-month-old rats (*14*). We examined the effect of age on renal cell $^{45}Ca^{2+}$ efflux and the responsiveness of the system to PTH. Figure 2 shows that Na^+-dependent $^{45}Ca^{2+}$ efflux did not differ in cells from the different aged rats. In contrast, the PTH responsiveness of the transport system was age dependent. When cells were pretreated with PTH, Na^+-dependent $^{45}Ca^{2+}$ efflux in cells from 6-month-old rats increased from 10.1 ± 1.1% to 16.0 ± 1.2%, a stimulation of 58%. With cells from 24-month-old rats, $^{45}Ca^{2+}$ efflux was 8.9 ± 1.8% without PTH and 10.6 ± 1.5% with PTH, a change of 19%, which was not statistically significant. These findings indicated that sensitivity of the renal cell Na^+-dependent $^{45}Ca^{2+}$ efflux system to PTH was blunted in the senescent rat. This age-related defect was specific since the forskolin-sensitive Na^+-dependent $^{45}Ca^{2+}$ efflux remained unchanged by the aging process.

Effect of Age on PTH-Stimulated Adenylate Cylase Activity in Renal Cell

This loss in the responsiveness of $^{45}Ca^{2+}$ efflux to PTH with age was in accord with the finding of a decrease in PTH stimulation of adenylate cyclase in membranes prepared from senescent rats. Table 1 shows that PTH stimulated the adenylate cyclase activity 3.40-fold with membranes from the young

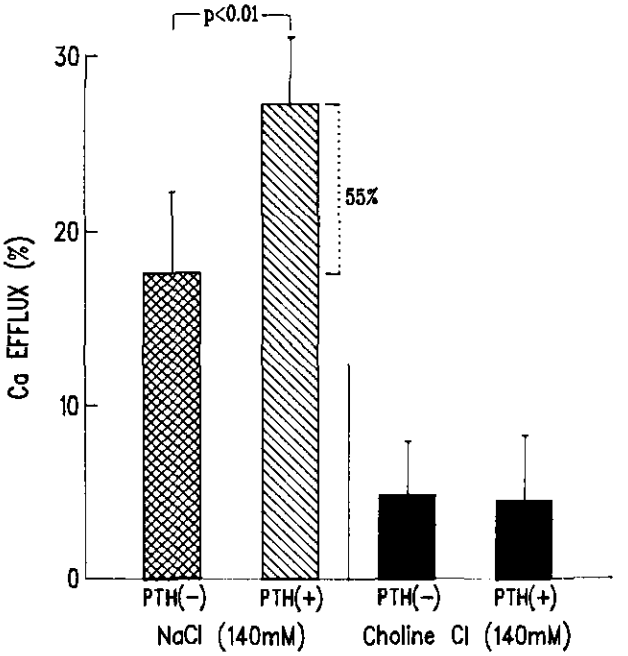

FIGURE 1. The effect of parathyroid hormone on $^{45}Ca^{2+}$ efflux from cells in the presence of extracellular Na^+ or choline$^+$.

animals but only 2-fold with the membranes from the aged rats. In the presence of GTP (1 μm), which by itself had little effect, PTH increased adenylate cyclase activity 6.68-fold and 3.36-fold in membranes from young and old rats, respectively. In contrast,

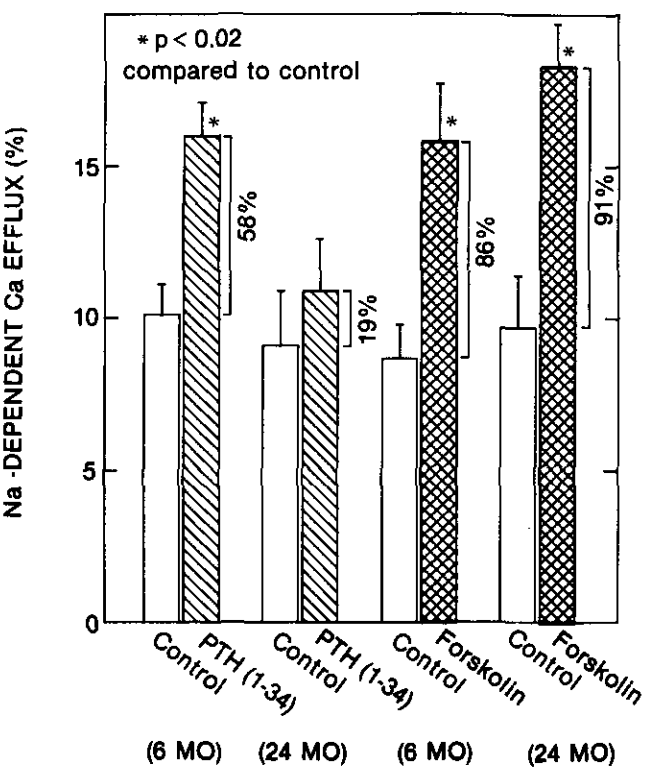

FIGURE 2. Effect of age of the rat on the sensitivity of Na^+-dependent $^{45}Ca^{2+}$ efflux to PTH and forskolin.

Table 1. Effect of PTH and age on adenylate cyclase activity in renal cell membranes.

Hormone	Relative activity	
	6 months	24 months
Basal	1.00	1.00
PTH	3.40 ± 0.15	2.00 ± 0.21
GTP	1.33 ± 0.15	1.54 ± 0.20
PTH ± GTP	6.80 ± 0.82	3.36 ± 0.41
Forskolin	13.2 ± 1.5	9.9 ± 1.3

stimulation of adenylate cyclase by forskolin did not change significantly with age.

Effect of Age on PTH Receptor in Renal Cell

One of the possible mechanisms for the blunted responsiveness to PTH in the senescent rats is at the hormone receptor level. Since PTH receptor was localized in the basolateral membrane, we examined the binding of PTH to receptors in the membranes isolated from young and old rats. A Scatchard plot analysis of the binding of ^{125}I-PTH to basolateral membrane showed that PTH binding site was 90 and 40 fmole/mg protein, respectively, in membranes prepared from young and aged rats. The affinity of the hormone to the receptor was not altered with age.

Effect of Age on GTP-Binding Proteins in Renal Cells

Agonist stimulation and inhibition of adenylate cyclase are known to involve GTP-binding proteins that couple receptors to the catalytic unit. Cells possess two classes of GTP-binding proteins that are associated with stimulation (Gs) and Inhibition (Gi) of adenylate cyclase by an agonist (*15*). Both Gs and Gi are oligomeric proteins with distinct α subunit that can be ADP-ribosylated with cholera and pertussis toxin, respectively, in the presence of NAD. Membranes prepared from renal cells isolated from 6- and 24-month-old rats were incubated with [α-^{32}P]NAD and either cholera or pertussis toxin to identify the α subunits of Gs and Gi.

An autoradiogram of the [^{32}P]ADP-ribosylated proteins with cholera toxin showed two bands of 45 and 52 kDa in membranes corresponding to the two forms of αs (*15*). With pertussis toxin, a band of 41 kDa was seen corresponding to the αi. To quantitate αs and αi, the appropriate regions of the slab gels were cut and radioactivity determined. The label of 45 kDa and 52 kDa, α subunits of Gs, was decreased by about 50% in membranes prepared from senescent rats as compared to young rats. Radioactivity in 41 kDa region, corresponding to α subunit of Gi, was decreased by 25% in membranes from aged rats.

Effect of Parathyroidectomy (PTX) on Age-Related Blunting of Responsiveness to PTH in Renal Cell

One of the features associated with senescent rats is an elevated level of immunoactive PTH. In the rats used in this study, the serum iPTH concentrations in 6- and 24-month-old rats were 100 ± 4 and 153 ± 19 pmole/L, respectively. We tested the hypothesis that the increased serum iPTH in rats contributed to the desensitization of the renal cells to PTH. Therefore, 6- and 24-month-old rats were PTX or sham operated, and the responsiveness of isolated renal cells to PTH was examined 48 to 72 hr after surgery.

With cells from sham-operated rats, the basal Na^+-dependent $^{45}Ca^{2+}$ efflux was about 10% for both ages. In PTH-treated cells from 6- and 24-month-old rats, the Na^+-dependent $^{45}Ca^{2+}$ efflux was increased to 15.9 ± 10% and 12.5 ± 1.0%, respectively. After PTX, Na^+-dependent $^{45}Ca^{2+}$ was slightly decreased in young rats. The incubation of cells from PTX 6-month-old rats with PTH increased the $^{45}Ca^{2+}$ efflux to 15.0 ± 1.1%; this was not different from the sham-operated animals. With cells from aged animals, PTX did not alter the basal level of Na^+-dependent $^{45}Ca^{2+}$ efflux. Incubation of PTH with cells from PTX 24-month-old rats resulted in an efflux activity of 16.4 ± 1.3%, a level higher than that of sham-operated 24-month-old rats, but comparable to the efflux found in cells from 6-month-old rats. The result demonstrated that PTX of 24-month-old rats completely reversed the decrease in PTH-sensitive Na^+/Ca^{2+} exchange activity. The response of the cells to forskolin, which also stimulated Na^+-dependent $^{45}Ca^{2+}$ efflux, was not significantly influenced by age nor by PTX.

Measurement of adenylate cyclase activity in renal membranes provided additional evidence that desensitization to PTH in senescent rats was reversed by PTX. In sham-operated 6- and 24-month-old animals, stimulation of adenylate cyclase by PTH + GTP were 3.86- and 2.10-fold, relative to the basal activity, respectively. After PTX, PTH activation did not change in membranes from 6-month-old rats, whereas the activation in membranes from 24-month-old rats was increased to 3.55-fold, a value not different from that of young rats.

The involvement of GTP-binding proteins in the reversal by PTX of the age-related desensitization to PTH was also suggested from experiments examining [^{32}P]ADP-ribosylation of αs and αi. In cholera toxin-catalyzed ADP-ribosylation of 45 and 52 kDa proteins, the two subunits of αs, radioactivity was reduced by 50% in the membranes from sham-operated aged rats, as compared to sham-operated young rats. After PTX, this decrement was changed to less than 25% which represented a partial recovery of αs. Autoadiograms of pertussis toxin-treated membranes show the decrease in ^{3}P label (approximately 30%) at

41 kDa in membranes from 24-month-old rats, relative to 6-month-old sham animals. This difference was largely negated after PTX.

The effect of PTX on PTH receptor site was also measured. In basolateral membranes prepared from both sham-operated or PTX 6-month-old rats, PTH binding site was about 90 fmole/mg protein. However, in membrane prepared from PTX 24-month-old rats, PTH receptor site was increased to 53 fmole/mg protein, as compared to 40 fmole/mg protein in membranes from sham 24-month-old rats. PTX did not alter the affinity of hormone receptor to PTH in both age rats.

Conclusion

The present study demonstrated that PTH, acting *in vivo* and *in vitro* increased renal Na^+/Ca^{2+} exchange activity. The specificity of the action by PTH was indicated by the finding that PTH (*1-84*) and synthetic PTH (*1-34*) increased Na^+-dependent Ca^{2+} efflux, whereas the biologically inactive PTH (*3-34*) did not. Cyclic AMP analogs and forskolin were also effective in enhancing Na^+-dependent Ca^{2+} efflux activity. PTH-sensitive Na^+-dependent Ca^{2+} efflux was markedly reduced in renal cells isolated from senescent rats as compared to young rats. PTH-stimulated adenylate cyclase activity was also decreased in aging. In contrast, forskolin-stimulated Na^+-dependent Ca^{2+} efflux and adenylate cyclase were not changed with age. These findings suggested a defect distal to cAMP generation in the senescent rats.

Binding of [^{125}I]PTH to the basolateral membrane indicated a 50% reduction of PTH receptor sites in membranes prepared from old rats. By using cholera and pertussis toxins as probes to quantitate the stimulatory (Gs) and inhibitory (Gi) GTP-binding proteins in adenylate cyclase complex, significant decreases in both GTP-binding proteins were found in cells from aged rats. When the aged rat was PTX, the decrease in PTH-sensitive Na^+-dependent Ca^{2+} efflux and adenylate cyclase activation by PTH could be completely reversed. The decrements in GTP-binding proteins and the decrease in PTH receptor site were partially reversed. Therefore, we can conclude that the age-related blunting of the responses of renal cells to PTH was due, at least in part, to the elevated serum level of iPTH in senescent rats.

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